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An Analysis of the Heavy Metal Content of the Scales of Several Fishes in Southwestern Kentucky

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AN ANALYSIS OF THE HEAVY METAL CONTENT OF THE SCALES OF SEVERAL FISHES IN SOUTHWESTERN KENTUCKY

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Thomas E. Dahl

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AN ANALYSIS OF THE HEAVY METAL CONTENT OF THE

SCALES OF SEVERAL FISHES IN SOUTHWESTERN KENTUCKY

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AN ANALYSIS OF THE HEAVY METAL CONTENT OF THE

SCALES OF SEVERAL FISHES IN SOUTHWESTERN KENTUCKY

Thomas E. Dahl May, 1978 38 pages

Directed by: R. Hoyt, G. Dillard, R. Prins, and J. Riley

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Scales of the stoneroller, *Campostoma anomalum* (Rafinesque), common shiner, *Notropus cornutus* (Mitchill), and the bluntnose minnow, *Pimephales notatus* (Rafinesque) were analyzed by means of atomic absorption spectrophotometry to determine levels of cadmium, clacium, copper, iron, lead, manganese, nickel and zinc. Metal concentrations were determined seasonally and relationships established between scale metal content and environmental water metal levels.

Calcium, iron, manganese and zinc were found in all samples analyzed. Cadmium, copper, lead and nickel were not observed in measurable quantities.

Metal concentrations varied interspecifically, but most showed little fluctuation in response to increased metal content of the water. Elemental composition of the scales was found to have been selective in that zinc and manganese were more readily taken up, even when present in considerably lower concentrations in the water. The metal content of scales was found to be related to behavioral characteristics of the species and the size of the fish.

INTRODUCTION

In recent years the role of heavy metals in the aquatic ecosystem has received increased attention. While most metals can and do occur naturally in the aquatic environment, their extensive use for industrial activities can result in abnormally high concentrations having important biological implications (Michigan Water Resources Commission, 1972).

It has been shown (Christensen, 1971; Brungs *et al*., 1973; Kleinert *et al*., 1974) that certain heavy metals are taken up and assimilated into some organs, tissues and even blood of given fish species. However, while considerable information has been gathered regarding soft tissues, relatively little information has been reported which establishes heavy metal uptake and incorporation within bony tissues of fish.

Van Coillie and Rousseau (1974) have shown by electron microscopic analysis that the scales of the white sucker, *Catostomus commersoni*, contain considerable concentrations of numerous heavy metals. These authors and Williams, *et al*., (1974), have inferred that a relationship exists between the metal content of the water and the concentration within the aquatic organisms therein. The latter, (1974), further notes;

> ". . . assimilation of metals by aquatic organisms will generally act to reduce their concentration in the waters surrounding the organisms and will increase the concentration of metals in the sediments that ultimately receive the biological material in which the metals have been concentrated."

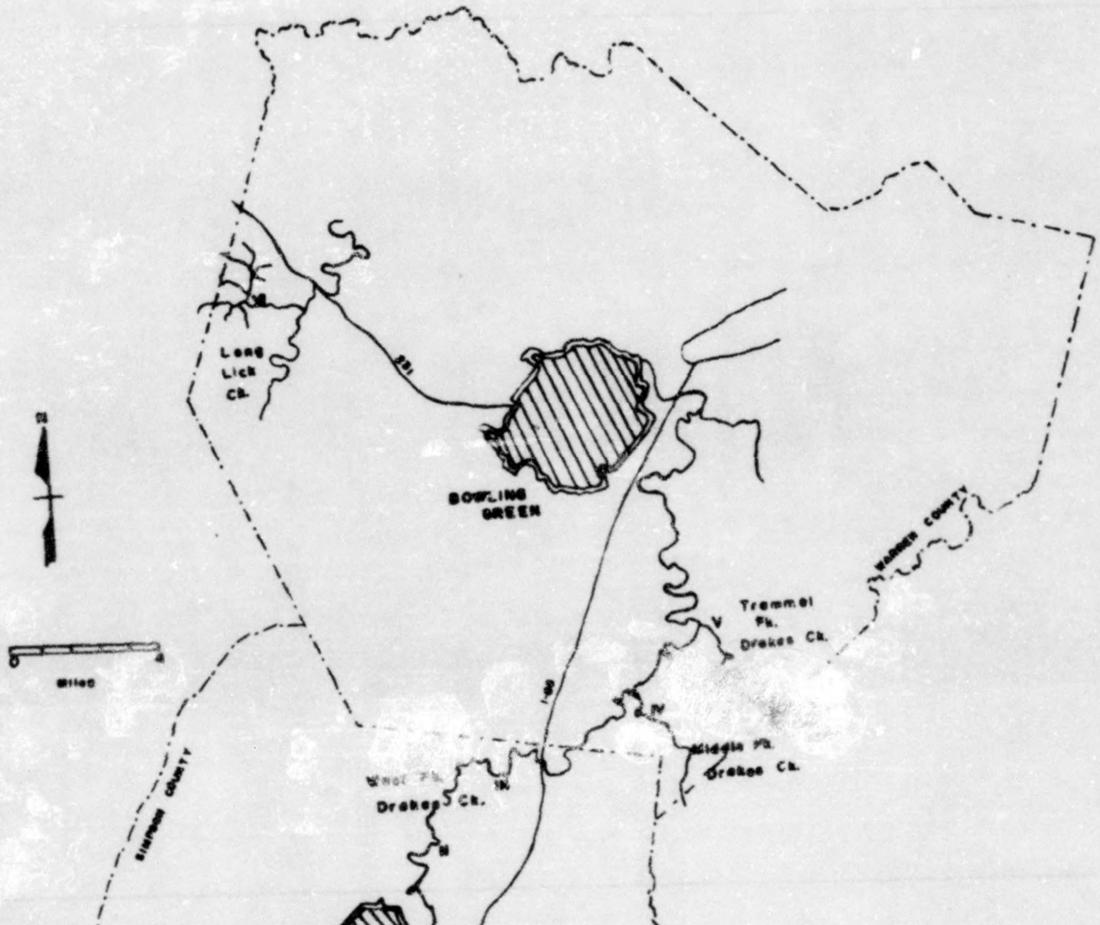
This study is a survey of the heavy metal composition of the scales of the stoneroller, *Campostoma anomalum*, common shiner, *Notropus cornutus*, and the bluntnose minnow, *Pimephales notatus*. It was designed to determine seasonal relationships between heavy metals in the water and their composition and concentration in fish scales.

STUDY AREA

A total of six collecting stations was established on four different stream systems in southwestern Kentucky (Figure 1). Fish were collected from sampling points which represented a variety of environmental conditions. These included the West Fork, Middle Fork and Trammel Fork of Drake's Creek and Belcher Creek in Simpson and Warren Counties, Kentucky. This region is characterized by a karstic topography with underlying formations of limestone, sandstone, shale and chert.

The West Fork of Drake's Creek is an order four stream (Horton, 1945) traversing agricultural areas and skirting the community of Franklin, Kentucky. It receives various inputs of partially treated domestic and industrial sewage via an outfall of the Franklin sewage lagoon system. Stations 1, 2 and 3 were established along the West Fork. Station 1 was located above Franklin, 0.40 km south on highway 73 in Simpson County, Kentucky. These waters received no apparent source of additives; however, construction of a bridge across the stream increased turbidity and leaching of sediments along the banks. Station 2 was situated at the highway 1171 bridge, 1.6 km below the Franklin municipal sewage lagoon and outfall. Waters of

Figure 1. Map of the West Fork, Middle Fork and Trammel Fork of Drake's Creek and Belcher Creek at the Long Lick Creek junction, showing location of collection sites.



Long
Lick
Ck.
BOWLING
GREEN
Trammel
Fk.
Drakes Ck.
Drakes Ck.
Drakes Ck.
FRANKLIN
Miles

this station were obviously discolored during the spring and summer months of the year and growths of algae and fungi were noticeable along the banks. Station 3 was at the bridge off Cedar Bluff Road, 11.3 km downstream from the sewage source. Although this area was surrounded by woodlands, evidence of discarded appliances and other debris was apparent.

Station 4 was located on the Middle Fork of Drake's Creek. The Middle Fork is an order five stream and is utilized for recreation as well as irrigation. The sampling site was located in Warren County, Kentucky, 0.80 km south of highway 240.

The Trammel Fork of Drake's Creek is an order five stream. Station 5 was located 1.2 km west of Alvaton, Kentucky, on the Lebanon Road. Stream waters were characterized by fast flowing riffles and deep pools with numerous downed trees and shrubs. No evidence of dumping or stream abuse was observed.

Station 6 was located on Belcher Creek, an order three stream in the western portion of Warren County. The site was located 3.6 km southwest of highway 231 on Old Hadley Road. Agricultural enterprises surrounded the drainage area. Some repair and minor channelization of the stream bed was known to have taken place to improve the ford.

MATERIALS AND METHODS

Field collections were made at quarterly intervals beginning in late February, 1977, and concluded in December, 1977. Fish were taken by seining and/or electroshocking. At no time during the collection procedure were fish exposed to waters other than those of their native collecting station.

Total length (TL) and numbers of individuals collected were recorded for each species per station per quarter. Specimens were fixed in a 10% formaldehyde solution until the time of scale removal. Generally an effort was made to collect five to seven stonerollers, 100 mm TL, five to seven common shiners 100-120 mm TL, and ten to twelve bluntnose minnows 60-70 mm TL. However, due to unusually severe climatic conditions during the winter of 1977, adult bluntnose minnows were not readily available, having apparently experienced an extremely high winter mortality. Young-of-the-year specimens were available in late spring and summer and were subsequently used. Lengths of these fish at most stations averaged between 30 mm and 50 mm total length. Where insufficient weight of scales was obtained, final concentration of metals was computed on a 2 g sample basis.

Prior to the removal of the scales, fish were vigorously rinsed with deionized, distilled water in an attempt to remove mucus and debris from the surfaces. The scales were then removed by scraping the body with a scalpel and placed on a pre-weighed glass slide. Where available, larger specimens were selected over smaller specimens. Scales were selected with no regard for location on the body or the regenerative condition. Two grams wet weight of scales constituted a minimal representative sample.

With minor modifications, scales were prepared for analysis using the acid digestion process for the determination of total heavy metal content as described by the Environmental Protection Agency (U.S.E.P.A., 1974). The technique modifications involved the following: A 2.0 g sample was combined with 25 ml deionized, distilled water and 3 ml concentrated nitric acid (HNO_3), placed on a hot plate and evaporated to dryness. Caution was used to prevent boiling of the sample solution. An additional 3 ml concentrated nitric acid were added to the precipitate and evaporated, followed by yet another 3 ml nitric acid, yielding a powdery residue when completed. Five ml concentrated hydrochloric acid (HCl) were then added, dissolving the final precipitate, and the resultant solute slightly warmed. Sufficient deionized, distilled water was added to bring the total volume to 25 ml, completing the sample preparation.

Water samples were taken at the time of quarterly fish collections. Approximately 1 liter of water was collected in a pre-acid-washed glass container and the pH immediately adjusted to 2.0 by additions of concentrated nitric acid (Bately and Gardner, 1977). Water temperature and dissolved oxygen were measured at the collection site. An additional 100 ml water sample was returned to the laboratory for pH and alkalinity determinations.

In the laboratory, 3 ml concentrated nitric acid were added to 100 ml of sample water and evaporated. Since water samples were relatively free of suspended materials, the addition of nitric acid increments was repeated only twice. Five ml concent[illegible]loric acid were then added, warmed and bro[illegible] volume of 25 ml by sufficient deionized [illegible].

A 100 ml volume of deionized [illegible]istilled water was treated similarly to serve [illegible] an analysis reagent blank.

Where the concentration of an element was too high, the sample was diluted by additions of deionized, distilled water. The original concentration was then calculated by using the formula:

Conc. final x Vol. final = Conc. original x Vol. original

In the analysis of calcium, all samples had to be diluted and a lanthanum oxide solution (29 g La_2O_3 + 250 ml concentrated HCl diluted to 500 ml with deionized water) was added in the ratio of 1.0 ml to 10.0 ml of solution.

Lanthanum oxide is used as an ionization suppressor and release agent in the analysis of calcium.

Calibration standards for each metal studied were prepared from standard stock solutions. Plots of absorbance versus concentrations of the calibration standards were then used to determine the concentration of metal ions in the samples. Since most metal concentrations exhibited a non-linear relationship at high concentrations, values for some metals were given simply as being greater than the highest known standard concentration used for analysis.

All measurements were made with a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer.

RESULTS

Metal Analysis. Of the eight metals tested, calcium, zinc, manganese and iron were found in measurable concentrations throughout the study period. Cadmium, copper, lead and nickel were present in only trace amounts or in undetectable amounts.

Calcium represented the greatest concentration of those metals observed throughout the study (Table 1). Other metals in order of greatest concentrations were zinc, manganese and iron.

Species Analysis. Scale metal concentrations were greater than station water samples for every metal recorded except iron in the spring and fall (Table 2). Common shiner scales exhibited the greatest metal composition by having the highest average calcium and zinc concentrations and the second highest manganese and iron levels (Table 2). Stonerollers ranked first in manganese, second in calcium and zinc and third in iron. Bluntnose minnows ranked last among the species tested in having the lowest levels of calcium, zinc and manganese, and even lower iron levels than station water samples.

Seasonal Analysis. Metal concentrations were observed to be greatest during the spring months when calcium, zinc and iron were present in their highest concentrations

Table 1. Average annual concentrations (mg/l) of calcium, zinc, manganese and iron in water samples and scales of the stoneroller, common shiner and bluntnose minnow.

Source	Ca	Zn	Mn	Fe
Water	198	0.1	0.6	5.5
Stoneroller	925	11.7	10.7	4.1
Common shiner	1010	13.4	7.3	4.3
Bluntnose minnow	338	5.2	3.7	3.5

Table 2. Average metal concentration (mg/l) by season in water samples and the scales of the stoneroller, bluntnose minnow and common shiner. Iron data were not available for the winter.

Metal/Source	Winter	Spring	Summer	Fall
CALCIUM				
Water	136	258	232	167
Stoneroller	517	1300	1090	792
Common shiner	645	1200	1220	972
Bluntnose minnow	508	349	199	296
ZINC				
Water	1.4	0.77	0.29	0.30
Stoneroller	6.9	15.0	14.6	10.3
Common shiner	10.7	15.7	13.9	13.3
Bluntnose minnow	4.7	5.6	4.2	6.0
MANGANESE				
Water	0.51	0.64	0.64	0.40
Stoneroller	6.5	16.0	14.5	5.8
Common shiner	8.6	5.7	10.0	4.9
Bluntnose minnow	6.8	2.9	2.0	3.1
IRON				
Water	-	6.4	3.7	6.3
Stoneroller	-	4.5	4.3	3.7
Common shiner	-	4.0	4.6	4.2
Bluntnose minnow	-	3.7	4.2	2.7

(Table 2). The summer months exhibited the highest level for manganese and the second highest levels for calcium and iron. Zinc ranked second during the fall months. The fall and winter months represented no noticeable trend regarding metal concentrations, other than having lower overall concentrations than the spring and summer months.

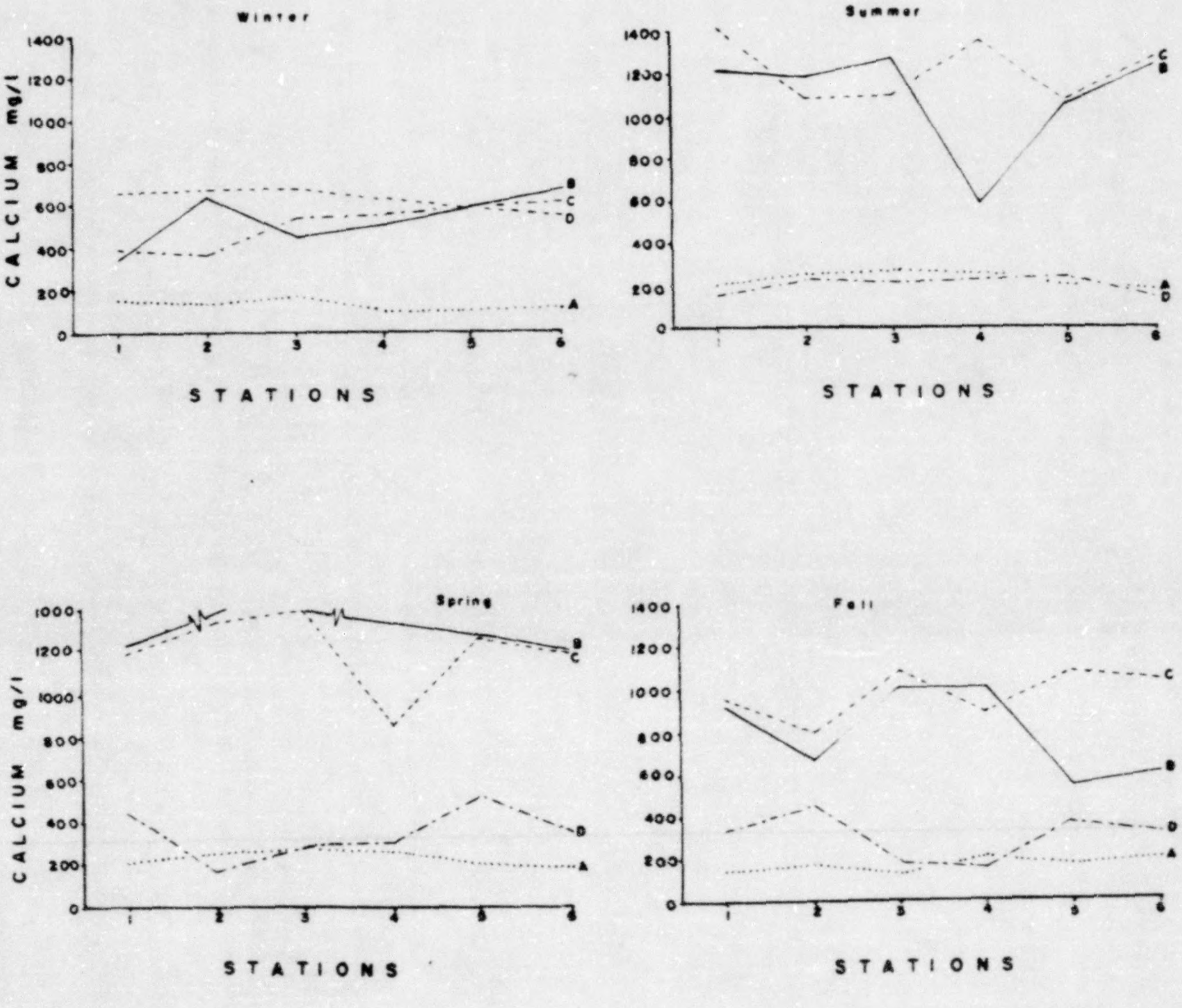
Station Analysis. When average annual metal concentrations were pooled for water samples and species studied, Station 6 had the highest collective metal levels, followed in order by stations 3, 2, 1, 5 and 4 (Table 3). In this ranking, Station 6 had the highest average zinc level and the second highest concentrations for calcium, manganese and iron. Station 3 had the highest average calcium and iron concentrations, but ranked third for zinc and fifth for manganese. Station 2 ranked first in manganese, second in zinc, third in iron and fifth in calcium. Station 4 had the lowest levels for calcium, zinc and manganese and ranked next to last for iron.

Calcium concentrations at each station showed unusual seasonal trends. During the winter, calcium levels in fish scales were present in only slightly greater amounts than water samples; however, during the remaining three seasons, common shiner and stoneroller scales showed markedly greater concentrations than bluntnose scales and water sample levels at all stations (Figure 2). Also, calcium levels of bluntnose minnow scales decreased from winter levels, approximating that of the water samples

Table 3. Average annual metal concentration (mg/l) in water samples and scales of the stoneroller, common shiner and bluntnose minnow at six collecting stations.

Metal/Source	1	2	3	4	5	6
CALCIUM						
Water	179	232	214	199	191	187
Stoneroller	838	965	1030	865	866	903
Common shiner	1170	967	1050	935	996	1050
Bluntnose minnow	304	215	304	339	429	356
ZINC						
Water	0.3	1.2	1.2	0.4	0.5	0.5
Stoneroller	9.9	13.9	12.3	8.5	10.2	13.0
Common shiner	11.7	14.1	14.2	10.7	12.4	17.3
Bluntnose minnow	6.2	5.5	4.5	3.8	4.8	6.2
MANGANESE						
Water	0.4	1.0	0.5	0.5	0.3	0.5
Stoneroller	11.5	15.3	8.6	6.6	8.8	13.4
Common shiner	6.5	11.3	4.4	6.4	6.7	8.6
Bluntnose minnow	6.5	7.1	2.3	1.4	1.7	3.3
IRON						
Water	4.8	5.3	10.1	3.7	1.9	7.1
Stoneroller	3.7	3.4	4.4	3.7	4.9	4.8
Common shiner	3.7	4.1	4.6	4.7	4.5	4.2
Bluntnose minnow	2.8	2.9	5.5	2.8	2.6	3.9

Figure 2. Concentration of calcium in milligrams per liter by season. A = concentration in station water samples, B = concentration in stoneroller scales, C = concentration in common shiner scales, and D = concentration in bluntnose minnow scales.



Winter
Summer
Spring
Fall
CALCIUM mg/l
STATIONS
1 2 3 4 5 6
0 200 400 600 800 1000 1200 1400
A
B
C
D

during the remaining seasons. Unusual calcium recordings were observed at Station 4 during the spring and summer when marked decreases occurred in common shiner and stoneroller scales, respectively.

Zinc was present in water samples in lower concentrations than scale samples at all stations throughout the study (Figure 3). Like calcium, bluntnose minnow scales had similar zinc levels to those of the stoneroller and common shiner at all stations during the winter but decreased appreciably during the remaining quarters (Figure 3).

Bluntnose minnow scales had higher levels of manganese [illegible] common shiners and stonerollers at Stations 1-3 [illegible] levels at Stations 4-6 during the winter (Figure [illegible] the exception of Stations 1 and 6 during the [illegible]nd Station 2 during the fall, bluntnose minnows [illegible] mang[illegible]ls than the other two species at [illegible]er of the study. Stonerollers had extrem[illegible] high manganese concentrations at Stations 2, 3 and 6 during the spring, averaging 16.0 mg/l for those stations.

Iron data were not available from any station during the winter. Iron concentrations showed the most unusual water sample versus fish scale metal levels of those metals tested. During the spring, water level concentrations were greater than scale levels at every station except Station 5 (Figure 5). However, during the summer, water sample levels were lower than scale levels at every station except

Figure 3. Concentration of zinc in milligrams per liter by season. A = zinc concentration in station water samples, B = concentration in stoneroller scales, C = concentration in common shiner scales, and D = concentration in bluntnose minnow scales.

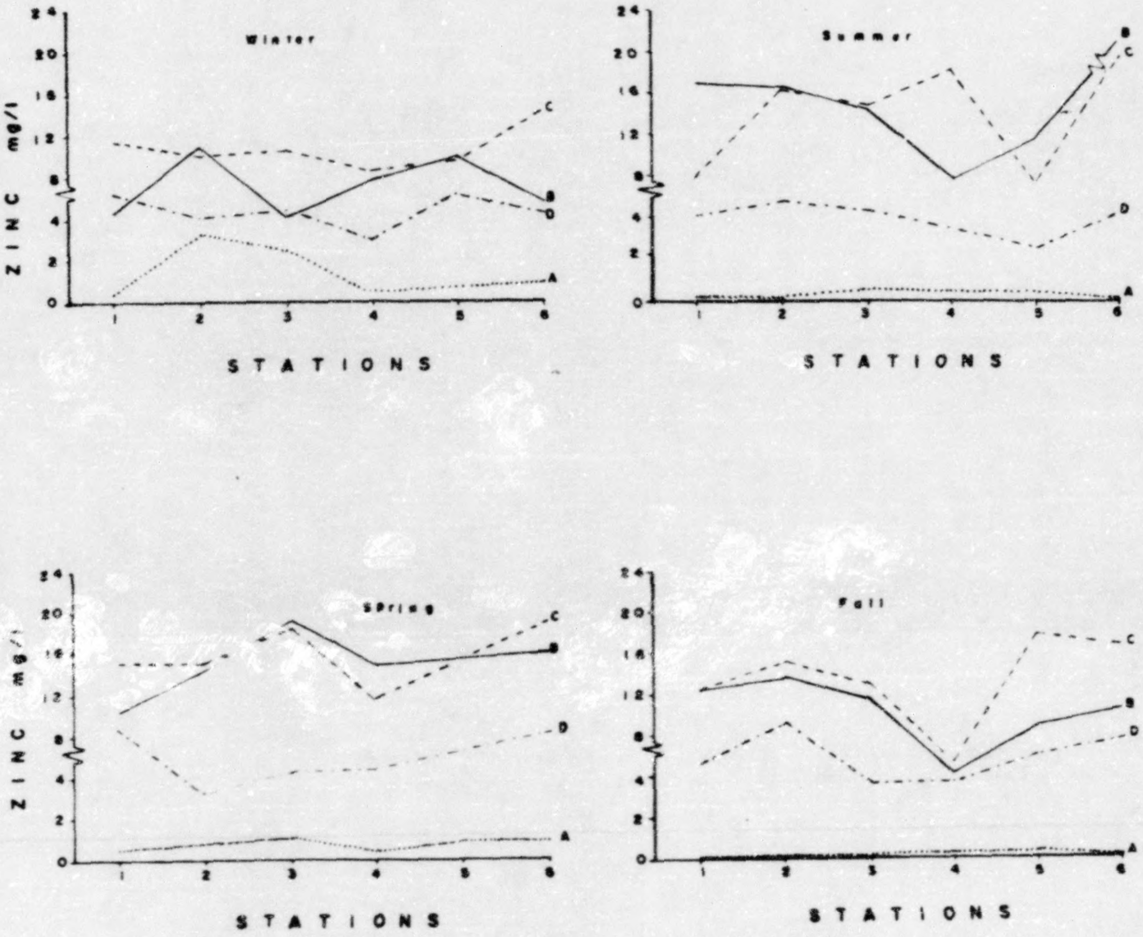

Winter
Summer
Spring
Fall
ZINC mg/l
STATIONS
A
B
C
D

Figure 4. Concentration of manganese in milligrams per liter by season. A = manganese concentration in the station water samples, B = concentration in the stoneroller scales, C = concentration in common shiner scales, D = concentration in bluntnose minnow scales.

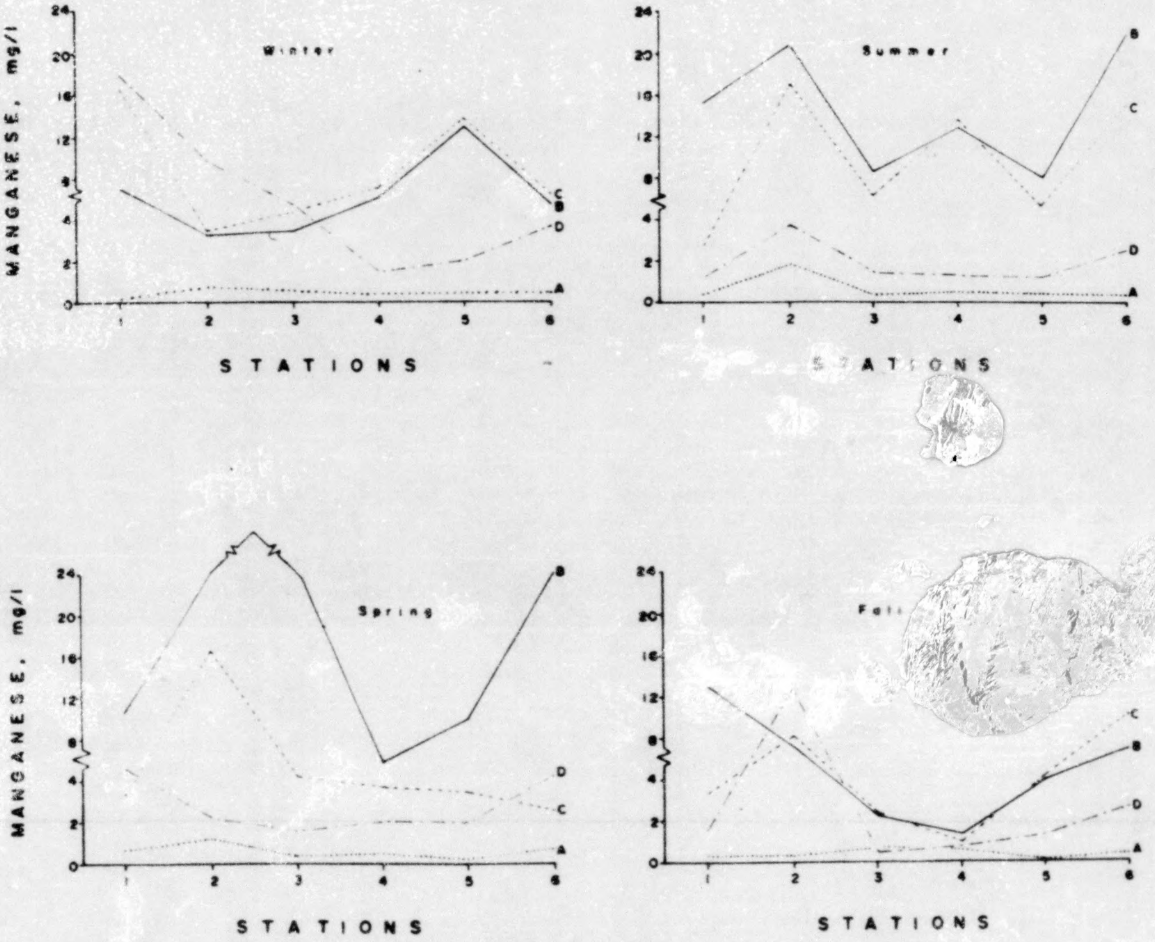
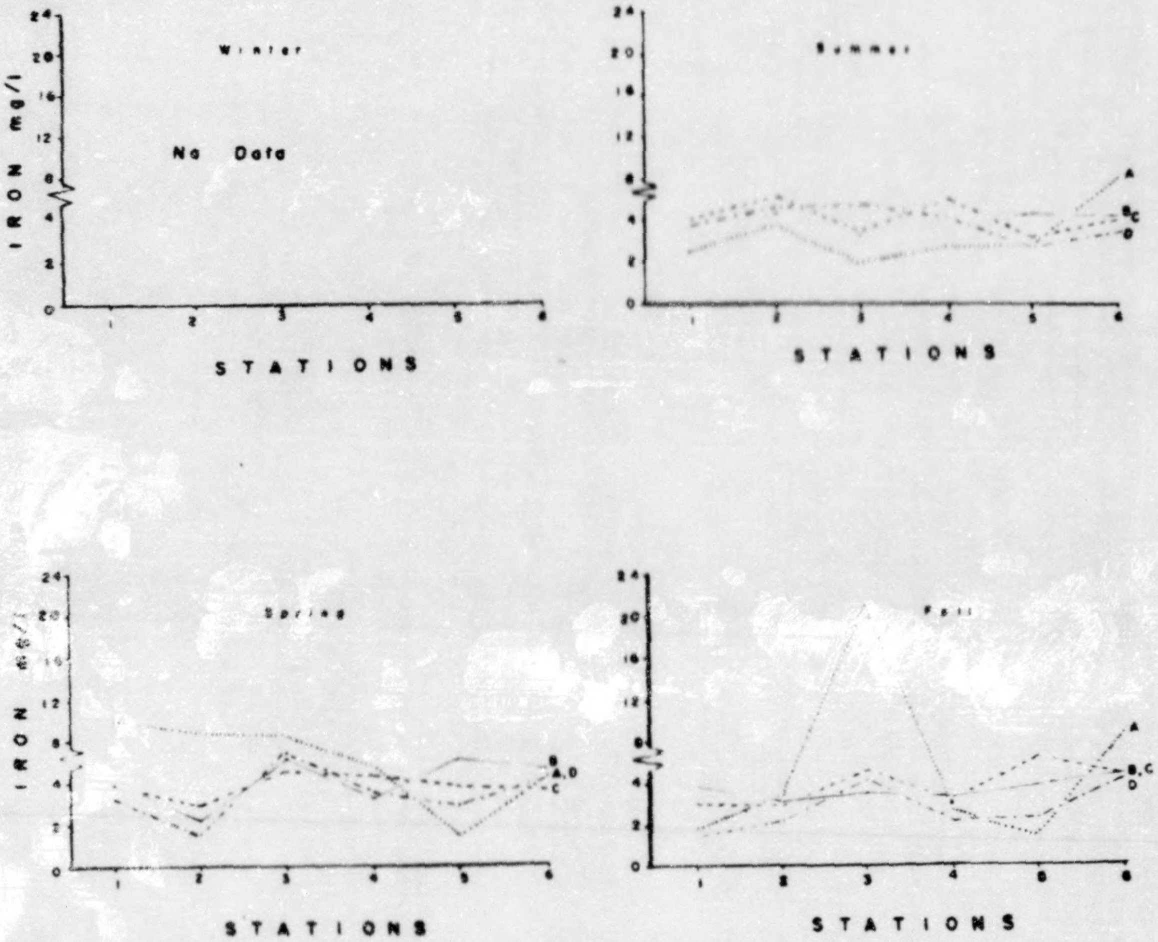

Winter
Summer
Spring
MANGANESE, mg/l
STATIONS
A
B
C
D

Figure 5. Concentration of iron in milligrams per liter by season. A = concentration in station water samples, B = concentration in stoneroller scales, C = concentration of iron in common shiner scales, and D = concentration in bluntnose minnow scales.


Winter
IRON mg/l
No Data
STATIONS
Summer
STATIONS
Spring
IRON mg/l
STATIONS
Fall
STATIONS

Station 6, while during the fall, concentrations were mixed, being higher than scale levels at some stations, and lower at others. An extremely high water sample iron reading, exceeding 20.0 mg/l, was observed at Station 3 during the fall.

DISCUSSION

Several specific factors can have significant effects on the amount of a chemical element found in a natural water system at any particular time. Types of drainage basins, increases in flow rate, amount of rainfall and natural or man-made inflow sources can greatly alter the overall concentration of materials in the water (Williams, et al., 1974). Extremes can occur not only seasonally but also in relation to intermittent sources of additives whether natural or otherwise.

Calcium is among the most variable elements found in fresh water (Macan, 1963). In previous studies calcium has been implicated in numerous ways in the growth and population dynamics of fresh water flora and fauna (Wetzel, 1975). However, the specific trends of calcium fluctuation may vary according to sample location, dependent upon intrinsic factors within the stream system.

Hynes (1970) has reported that waters flowing through areas of limestone or other calcareous rock deposits will undoubtedly contain high amounts of calcium. Thus, the levels of calcium observed in this study were not unexpected. The primary source of the calcium was probably the limestone rock composition of the drainage basin with supplemental sources such as the municipal sewage outfall on the West Fork of Drake's Creek at Franklin, Kentucky.

In this study, zinc was found in considerably higher concentrations than the .081 mg/l typical of the southeastern region of the United States (Durum, 1974). However, this was not totally unexpected since Leckie and James (1974) state that certain trace metals, including zinc, are commonly found in domestic waste waters in the mg/l range. Although the chief source of zinc detected in this study was probably the native strata making up the drainage basin, as reported by Durum (1974), it may well have been added by outside agents such as the sewage outfall at Franklin, Kentucky.

Zinc has been identified by several workers as having been present in scale structures (Cowgill, *et al.*, 1968; Van Coillie and Rousseau, 1974). When carp, *Cyprinus carpio*, were subjected to radioactive zinc chloride (Zn^{65}), zinc accumulation was rapid and irreversible. Zinc increases were also inhibited by higher calcium concentrations in the test waters (Lebedeva and Kuznetsova, 1969).

Manganese also forms an important component of the elements found in water. Morgan (1967) identified manganese as a relatively abundant element second only to iron among the heavy metals found in natural waters. However, Livingston (1963) found that concentrations of manganese in water bodies varied too greatly to establish a mean concentration of any significance. Manganese has also been described as being an essential element in nutrition and is found in every kind of plant and animal tissue (Morgan, 1967).

The amount of iron in water is determined largely by the physical chemistry which regulates bacterial metabolism within a system (Wetzel, 1975). Hem (1967) also points out that the exposure of reduced iron minerals to an oxidizing condition may bring large amounts of iron into solution. In this study, iron formed the second largest ionic component of water. The observed fluctuations in iron concentration in the different water systems was probably due to the enrichment of iron commonly found to occur in surface waters with high content of dissolved organic matter as pointed out by Wetzel (1975).

Although the water analysis data indicated the availability of four metals, it did not identify the relationship of these metals in actual metabolic activity. The role of an ion in metabolism, whether useful or toxic, depends entirely on the tissues and other ions present and in what proportion (Macan, 1963). Recent studies have indicated the importance of calcium, zinc, manganese and iron as essential micronutrients (Bowen, 1966). Without question, the results of this study indicate that scale metabolism does concentrate certain heavy metals. Metal levels in scales of all three species of fish were higher than water sample levels throughout the study, except for iron in the spring and fall quarters. Van Coillie and Rousseau (1974) reported similar findings involving a wide range of heavy metals by electron microscopic analysis of the scales of the white sucker, _Catostomus commersoni_.

The concentration of heavy metals in all three species of fish analyzed was observed to be dependent upon 1) interspecific differences and 2) size of the fish. Species differences were responsible for some subtle variations in the metal content of the scales, particularly on a seasonal basis. Although many factors could have been involved influencing the high average metal concentrations found in the scales of the common shiner, it would seem logical to attribute high metal content to the diet. Similarly, increases in zinc and manganese in the scales of the stoneroller, during the spring and summer, were believed to have been brought about by the feeding behavior of the species.

The common shiner is generally considered an aggressive omnivore (Carlander, 1969). The stoneroller, however, is a bottom feeder, taking in large quantities of sediment and debris (Clay, 1962). The convention of equating organismal heavy metal content to that of the water can be misleading. Organisms do not necessarily obtain all heavy metals directly from the water but perhaps from other sources such as food and the sediments (Burrell, 1975). As feeding activity increased during the spring months, the stoneroller ingested sediments either as food or incidentally with the subsequent incorporation of the metals into active scale metabolism. The result was increased concentrations of all metal salts within the scales. Manganese content of the waters did not correspond to the sizable increases of metal in the scales of the stoneroller during the spring and summer quarters. Due to the chemical aspects of the metal, manganese was probably heavily concentrated in the sediments of streams (Michigan Water Resources Commission, 1972); thus, it was to be expected that the feeding behavior of the stoneroller would result in ingestion of large quantities of the metal, explaining its high concentration. The release of elements from the sediments to the water column was uninterrupted. Water analysis provided only an indication of increases or decreases in heavy metal concentration occurring in solution at a specific point in time. It could not account for the biology of the species and the interactions with the environment. The fact that such large increases in manganese content of the scales occurred characteristic to a particular species was important. It identified the method of uptake as having been behaviorly related. It also indicated that elemental composition of the scales was not static.

While stonerollers and common shiners maintained fairly consistant average concentrations of metal salts within their scales, the bluntnose minnow scales contained lower levels of the same elements. This was apparently due to the size of the fish used in the analysis. As a result of an extremely severe winter in 1977, the size of the bluntnose minnows collected was considerably different from the size of the stonerollers and common shiners. In many cases the size of the bluntnose minnow necessitated the use of less than a 2.0 g sample of scales for analysis. However, even when sufficient scales were available, the metal content remained low.

Iron was the only heavy metal found in higher concentrations in the water than in biological tissue. It was interesting to note that, based on the assumption that iron was present in the sediments, iron was not retained by the scales of the fish as were zinc and manganese. This ultimately lead to the conclusion that scales served as sites of selective metal incorporation.

Calcium, zinc, manganese and iron were present in all fish scales analyzed. While each element exhibited unique trends, these metals were selectively absorbed and retained by the stoneroller, common shiner and bluntnose minnow. Cadmium, copper, lead and nickel were not retained in measurable amounts. The latter elements were either totally lacking in the ecosystem or were not accumulated in the scales in great enough quantities to produce observable levels. It can be theorized that the scale formation process leads to the retention of specific metals or that scales act as the storage sites for some non-essential elements not actively used in metabolism and, in the process, act to provide some physiological resistance to their effect (Van Coillie and Rousseau, 1974).

Periodic metal increases and decreases in the scales were not a direct result of corresponding changes in mineral composition of the water. This was in direct opposition to the findings of Van Coillie and Rousseau (1974), who reported that as certain metals--including manganese--occurred in increasing concentration in the waters, other component metals would decrease in the scales. They believed that excess metal from the water could replace calcium in the middle of the osteoid structures, thus establishing a direct relationship between mineral composition of the scale and the surrounding aquatic environment. Heavy metal concentrations in the scales of any of the three fish species analyzed did not accurately reflect the environmental conditions. Rather, concentrations of elements seemed to be dictated by the activity and behavioral patterns of the fish on a seasonal basis. The most notable example of this was the proportional increases in metal content of the scales with the amount of sediment ingested by the stoneroller. Changes observed in the concentrations of calcium, zinc, and manganese were believed to have been brought about solely by the feeding behavior of the fish species.

Two essential features of this fluctuation process are evident. The trend apparently did not include iron since iron was found in some cases in greater quantities in the surrounding waters than in scales. Iron was also the only metal that did not change in proportion to increased sediment intake by the fish. Secondly, metal concentrations in scales have the ability to undergo dynamic fluctuations.

Metals such as zinc and manganese were gained and lost from scale structures on an annual basis; concentrations not being static.

Yearly high levels of calcium, zinc,and iron were observed during the spring quarter. In the water, this phenomenon could be attributed to increased water temperatures stimulating microbial activity and competition for elements such as zinc in the sediments. Subsequent cycling and release of the elements through the biota within several days time may explain the observed seasonal increases. Similar conclusions were presented by Leckie and James (1974). Increased metal content of fish scales in the spring may be explained by the fact that this particular time period represented the time of most active scale formation and growth (Carlander, 1969).

Manganese exhibited trends similar to those of the other metals by having elevated levels during the spring and summer quarters. It has been reported (National Academy of Science, 1973) that variation in manganese levels of water samples may be the result of increased materials in suspension, particularly in periods of high water during the spring. However, in this study manganese fluctuation was so slight that it could not be considered meaningful on a seasonal basis. Manganese increases in the scales were believed to have been related to increased sediment intake as discussed earlier.

On an individual station basis, measurements yielded some insight as to heavy metal concentrations which qualified some observations. No specific reason could be recognized as to why station 6 maintained the highest overall average metal concentrations. No apparent source of contamination was evident; therefore, the metal levels of station 6 must be attributed to combinations of factors identified as influencing heavy metal content at the beginning of this discussion.

The metal content of the waters and fish scales of stations 2 and 3 were probably affected by the input of materials from the Franklin sewage outflow. Station 2, immediately downstream from the effluent, contained notable amounts of manganese suspected of having been a product of the sewage system.

Calcium and zinc concentrations in the scales varied on a station to station basis in accordance with periods of active growth (seasons) and the availability of fish at a particular location. Lower average concentrations of calcium in the spring and summer quarters at Station 4 may be explained by the unavailability of sufficient numbers of fish for analysis.

Iron also showed some peculiar station tendencies. In the winter, iron was present in greater concentration in the waters of stations 1, 2 and 3 than in any of the fish species. This was believed to have been due to the lack of concentration of iron in the scales rather than other factors although inputs of iron from the sewage outflow may have contributed to this phenomenon. An apparent source of contamination of iron was noted at Station 3 during the fall when iron concentrations exceeded the 20.0 mg/l level.

SUMMARY

Scales of the stoneroller, common shiner and bluntnose minnow were analyzed by atomic absorption spectrophotometry to determine heavy metal content and fluctuation on a seasonal basis. The metal levels of the scales were compared to metal concentrations in natural waters.

All scales analyzed contained measurable amounts of calcium, zinc, manganese and iron. With the exception of iron, metal concentrations fluctuated on a seasonal basis characteristic to the behavioral patterns of the particular fish species.

Considerable additions and losses of heavy metals ions in the scales were believed to have been related to the sediment composition of the diet. Whether these observed changes in concentration were in response to metabolic requirements or incidental accumulation was unknown.

No synergistic affects on metal concentrations were observed. However, the size of the fish used for analysis was important in determining overall metal levels.

A direct relationship could not be established linking the amount of an element found in the scales with the proportional concentration of the same element in the environment.

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Appendix A. Concentration of calcium in milligrams per liter in water samples and scales of the stoneroller, common shiner and bluntnose minnow at six collecting stations.

Source	Winter	Spring	Summer	Fall
Water				
1	152	216.	205.	143.
2	138	363.	255.	171.
3	175	271.	277.	134.
4	101	231.	264.	202.
5	---	202.	205.	165.
6	112	266.	185.	186.
Stoneroller				
1	341.	1230.	1200.	918.
2	638.	1390.	1170.	662.
3	462.	1390.	1270.	1010.
4	510.	1340.	596.	1010.
5	594.	1280.	1050.	544.
6	557.	1200.	1250.	605.
Common shiner				
1	660.	1190.	1480.	956.
2	660.	1340.	1080.	790.
3	660.	1390.	1090.	1080.
4	640.	859.	1350.	889.
5	587.	1360.	1060.	1080.
6	660.	1200.	1290.	1040.
Bluntnose minnow				
1	383.	451.	154.	334.
2	365.	167.	242.	449.
3	539.	290.	211.	176.
4	554.	317.	---	145.
5	594.	519.	240.	361.
6	612.	352.	150.	310.

Appendix B. Concentration of zinc in milligrams per liter in water samples and scales of the stoneroller, common shiner and bluntnose minnow at six collecting stations.

Source	Winter	Spring	Summer	Fall
Water				
1	0.4	0.5	.1	0.0
2	3.1	---	.2	0.2
3	2.4	1.0	.5	1.0
4	0.4	0.5	.4	0.2
5	---	0.9	.4	0.3
6	0.8	0.9	.2	0.2
Stoneroller				
1	4.4	10.5	16.9	12.6
2	10.8	14.5	16.7	13.8
3	4.0	19.1	14.6	11.7
4	7.4	14.8	7.7	4.2
5	9.9	---	11.9	8.9
6	5.4	16.0	20.0	10.6
Common shiner				
1	11.5	15.1	7.6	12.5
2	10.0	15.1	16.4	15.1
3	10.5	18.5	14.7	13.1
4	8.4	11.4	18.1	5.0
5	9.3	15.3	7.6	17.7
6	14.2	19.0	19.3	16.6
Bluntnose minnow				
1	6.2	8.8	4.1	5.5
2	4.0	3.3	5.6	9.2
3	4.7	4.6	4.9	3.7
4	2.9	4.8	---	3.7
5	6.0	---	2.5	6.0
6	4.5	8.2	4.1	7.8

Appendix C. Concentration of manganese in milligrams per liter in water samples and scales of the stoneroller, common shiner and bluntnose minnow at six collecting stations.

Source	Winter	Spring	Summer	Fall
Water				
1	.2	.7	.4	.3
2	.8	1.2	1.8	.3
3	.6	.4	.3	.7
4	.4	.5	.5	.6
5	---	.3	.4	.1
6	.5	.8	.4	.4
Stoneroller				
1	7.0	10.6	15.3	13.0
2	3.3	30.0	10.8	7.1
3	3.5	20.0	8.7	2.2
4	6.2	5.9	13.0	1.4
5	13.2	10.0	8.1	4.0
6	5.7	20.0	21.0	7.0
Common shiner				
1	16.7	3.3	2.7	3.3
2	3.5	16.5	17.1	8.1
3	4.4	4.5	6.4	2.3
4	7.0	3.7	13.7	1.0
5	13.8	3.5	5.0	4.4
6	6.5	2.6	15.2	10.3
Bluntnose minnow				
1	18.0	5.2	1.2	1.5
2	9.7	2.2	3.7	12.7
3	5.6	1.6	1.4	.4
4	1.5	2.0	---	.7
5	2.0	2.0	1.2	1.4
6	3.7	4.4	2.5	2.7

Appendix D. Concentration of iron in milligrams per liter in water samples and scales of the stoneroller, common shiner and bluntnose minnow at six collecting stations.

Source	Winter	Spring	Summer	Fall
Water				
1	---	10.0	2.5	1.8
2	---	8.8	3.8	3.4
3	---	8.4	2.1	20.0
4	---	5.6	2.9	2.7
5	---	1.4	2.9	1.4
6	---	4.4	8.3	8.7
Stoneroller				
1	---	4.1	3.2	3.8
2	---	2.2	4.9	3.1
3	---	6.2	[illegible]	3.6
4	---	3.2	[illegible]	3.4
5	---	6.0	4.9	3.8
6	---	5.1	4.8	4.4
Common shiner				
1	---	4.0	4.1	3.0
2	---	3.0	6.3	2.9
3	---	5.0	3.6	5.2
4	---	4.7	6.2	3.3
5	---	3.8	3.4	6.4
6	---	3.6	4.4	4.7
Bluntnose minnow				
1	---	3.3	3.8	1.4
2	---	1.5	5.3	2.1
3	---	6.8	5.6	4.2
4	---	3.5	---	2.1
5	---	2.9	2.8	2.2
6	---	4.1	3.5	4.2